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P.W. Krauter
R. Martinelli
S. Martins

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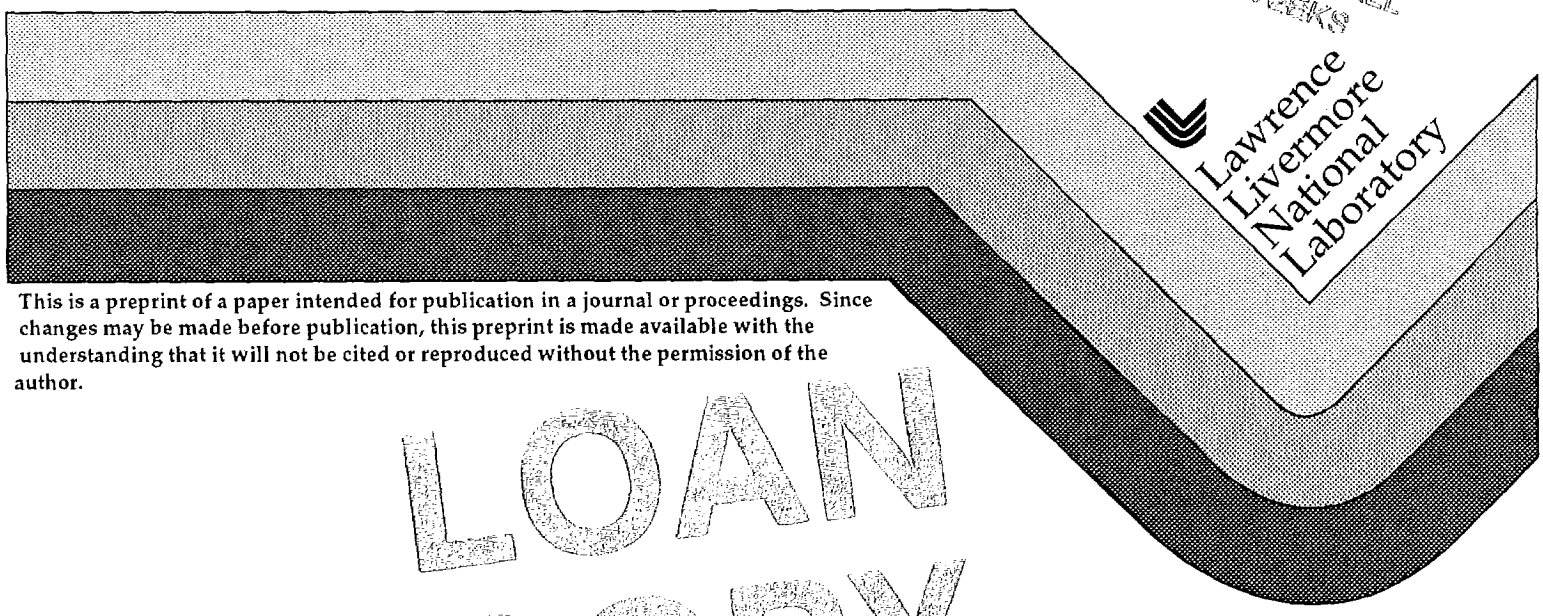

Title: Removal of Cr(VI) from ground water by *Saccharomyces cerevisiae*

Authors: P. Krauter*, R. Martinelli[1] & S. Martins

Environmental Protection Division, [1]Health & Ecological Assessment Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA (*requests for reprints)

Key words: *Saccharomyces cerevisiae,* bioreduction, chromium, ground water

Abstract

Chromium can be removed from ground water by the unicellular yeast, *Saccharomyces cerevisiae*. Local ground water maintains chromium as CrO_4^{2-} because of bicarbonate buffering, and pH and E_h conditions (8.2 and +343 mV, respectively). We used commercially available, nonpathogenic *S. cerevisiae* to remove Cr(VI) from ground water. Parameters, such as temperature, pH, glucose, and Cr(VI) concentrations, were also examined. *S. cerevisiae* removed Cr(VI) under aerobic or anaerobic conditions with a slightly greater rate occurring under anaerobic conditions. Our kinetic studies reveal a reaction rate (V_{max}) of 0.227 mg hr^{-1} (g dry wt. biomass)$^{-1}$ and a Michaelis constant (K_m) of 145 mg/l^{-1} in natural ground water using mature *S. cerevisiae* cultures. We found a rapid initial removal of Cr(VI) with freshly hydrated cells (55 to 67 mg hr^{-1} (g dry wt. biomass)$^{-1}$ in the first 2 minutes) followed by a much slower uptake (0.6 to 1.1 mg hr^{-1} (g dry wt. biomass)$^{-1}$) that diminished with time. A materials-balance for a batch reactor over 24 hours resulted in an overall shift in redox from +321 to +90 mV, an increase in the bicarbonate concentration (150 to 3400 mg/l) and a decrease in the Cr(VI) concentration in the effluent (1.9 mg/l to 0.0 mg/l).

Introduction

Metal-refining industries, mining operations, and manufacturing industries produce wastes that may contain heavy metals. Occasional leaching of the waste solids by rain percolation or surface water runoff has been a source of contamination of the ground and surface water. Chromium is a common contaminate. It is used in plating shops, tanneries, and aerospace facilities, and has been found in the effluent of facilities with water towers and steam generators as well as in mine tailings. Epidemiological data suggest that chromium compounds are carcinogenic in

humans (IARC 1973; Bidstrup & Case 1956; Shi et al. 1991). Studies provide evidence that hexavalent chromium [Cr(VI] salts of Na, K, and Ca are mutagenic (Venitt & Levy 1974).

Several species of bacteria, yeast, and algae are capable of accumulating metal ions extracellularly or internally to concentrations several orders of magnitude higher than the background concentration. *Pseudomonas* species have been studied and characterized for chromate reductase activity (Ishibashi et al. 1990). Although *P. aeruginosa* and *P. fluorescens* successfully removed Cr(VI) from ground water in studies conducted at LLNL (unpublished results), these organisms also taint the water with an unpleasant odor and *P. aeruginosa* is a human pathogen. For these reasons, we studied the safer microorganism, *Saccharomyces cerevisiae*, for its ability to bioreduce and remove Cr(VI) compounds from ground water.

S. cerevisiae is capable of accumulating Co^{2+} and Cd^{2+}(Norris & Kelly 1977), U (Strandberg et al. 1981), Cs, Sr, U (de Rome & Gadd 1991), Cu(II) (Huang et al. 1990). and Cr^{2+}, Co^{2+} and Cd^{2+} (Brady & Duncan 1994). Microorganisms respond to metals by a number of processes, including transport, biosorption to cell biomass, entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Gadd & Griffiths 1978; Gadd 1990a, b). Bioaccumulation of metal cations has been demonstrated (Norris and Kelly 1977; Brady and Duncan 1994) by two processes; an initial rapid accumulation that is independent of metabolism and temperature; and a metabolically mediated process that internalizes the cation into the cell. Energy-dependent uptake of divalent cations by *S. cerevisiae* is well known (Fuhrmann & Rothstein 1968; de Rome & Gadd 1991) with influx being dependent on the electrochemical proton gradient across the plasma membrane (Borst-Pauwels 1981).

The purpose of this study was to investigate the use of a fermentative microorganism, *S. cerevisiae*, as an agent for the removal of Cr(VI) (i.e. chromate) in contaminated ground water. In addition, we studied the effects on Cr(VI) removal rates of extra cellular factors, such as pH, temperature, and energy source concentration. Emphasis was placed on establishing chromate removal kinetics in batch cultures.

Materials and methods

We used borosilicate glassware, which has low metal cation binding properties for the laboratory studies. All glassware used for metal analyses was washed with detergent, rinsed, soaked in a 2-4% nitric acid bath, rinsed three times in ultra pure water (Millipore Milli-Q purification system), and autoclaved for sterility.

Ground water from well number MW-004 was used for this study. MW-004 ground water contains 32 µg/l Cr(VI), and it is typical of the slightly alkaline and oxidizing conditions in ground water at the Lawrence Livermore National Laboratory (LLNL) (Table 1). The ground water was vacuum filtered through Millipore 0.45 µm filters. This ground water was also contaminated with 44 µg/l trichloroethene (TCE), 2.4 µg/l chloroform, and 2.2 µg/l of 1,1 dichloroethene (DCE) (Table 2). These volatile organic compounds were removed in the filtration process. The ground water was not autoclaved because dissolved substances in the water form a floc when autoclaved.

Physical treatment of yeast cells

The organism used in this study was commercially produced, dehydrated *S. cerevisiae*. (Fleischmann's, Oakland, California). In shake flask experiments *S. cerevisiae* was cultured in Sabouraud broth (SAB) containing dextrose, 20.0 g/l; peptone, 10.0 g/l; and 1 l of ultra pure water adjusted to pH 7 with 2 M HCl and autoclaved 25 min at 121°C. *S. cerevisiae* cultures were regenerated by weighing pelleted cells into 2% SAB in shaker flasks and incubated overnight at 30°C on a 220 rpm rotary shaker. Cells were harvested the next day by centrifuging in 200-ml Kimax centrifuge bottles for 10 min at 2500 rpm. The resulting cell pellet was then suspended in filtered ground water and centrifuged again for 10 min at 2500 rpm. The water was decanted off the pellet and cells were resuspended in sterile, filtered ground water. Final cell density was about 8 g dry wt biomass/l.

In the reactor studies, the dehydrated yeast pellets were weighed and added directly to the test vessels to an initial concentration of about 8 g/l. Dried cell weights were obtained by filtering a known volume of the culture through a tarred, 0.45-µm membrane filter; rinsing the resulting pellet; and drying it at 80°C for 24 h.

The pH was monitored in reactor tests with an Ingold OPM 131 electrode (New Brunswick Sci. Co., Edison, New Jersey). For experiments with live cells, the pH was maintained at 6.5 by neutralizing the acid production with 4N NaOH. A strong alkali was chosen to limit dilution; cells in localized areas in the reactor may have died because of the strong base addition.

Cells were killed by three different methods; autoclaving them at 120°C and 2 ATM for 35 min, or irradiating them with Cs^{137} for 90 min, or by adding mycostatin to the culture. Because autoclaving was the most reliable method of killing yeast cells, it was used for all subsequent experiments. Triplicate plating on SAB plates was used to verify that dead cells were nonviable.

Analytical methods

Measurements of redox potential were used to determine the oxidizing or reducing capability of the reactor fluid. Quinhydrone (Sigma Chemical Co., St. Louis, Missouri) was used to calibrate the E_h probe (Orion Ionalyzer combination redox probe). The dissolved oxygen was measured by an oxygen probe (Ingold, polargraphic). The redox potential readings were reported relative to the normal hydrogen electrode, corresponding to the filling solution used and the temperature of the solution measured.

Glucose was provided as the carbon and energy source. The Cr(VI) source was $K_2Cr_2O_7$ (Sigma Chemical Co. St. Louis, Missouri). To distinguish the biotic from abiotic Cr(VI) removal, tests were run with chemical control (CC) series, dead-cell (DC) control series, and the live-cell (LC) test series.

Hexavalent chromium samples were collected in 25-ml aliquots in acid-washed, screw cap, 50-ml test tubes and analyzed spectrophotometrically using modified U.S. Environmental Protection Agency (EPA) methods (Standard Method, 17th ed. 3500-Cr D. Colormetric method). This method was based on the reaction of Cr(VI) with diphenylcarbazide in an acidified solution. Measurements were conducted in 5-cm path length cuvettes with a Shimadzu spec UV160U at 540 nm absorbency under yellow room lighting for light-sensitive reagents.

Total chromium was analyzed on a Perkin-Elmer 5100 series Atomic Absorption Spectrophotometer (AAS) equipped with a 4-in. single-slot flame head. The AAS was outfitted with a Transverse High Temperature Graphite Furnace and AS-71 autosampler. A Perkin-Elmer chromium lamp was used as the energy source for both flame and furnace analyses. Biomass was collected on 0.45-μm Millipore filters and digested in a solution of 50:50 nitric:hydrochloric acid for 24 to 48 h, depending on the digestion progress.

Determination of parameters affecting kinetics of process

Effect of pH on chromate removal

Ground water pH was adjusted to 7 different pH levels with 2N HCl from pH 8.2 to 2.0 in 250-ml Erlenmeyer flasks. Each flask initially contained 1.5 g dry wt biomass/l, 50 mM glucose, and 2 mg/l Cr(VI) and was incubated for 24 h at 20°C. A duplicate set of flasks was inoculated with 1.5 g dry wt biomass/l of dead yeast and handled in the same manner. The cell suspensions were filtered through 0.45-μm membrane filters, and replicate aliquots of the supernatant were analyzed for Cr(VI).

Effects of temperature on chromate removal

Flasks containing filtered MW-004 ground water and 25 mM glucose were inoculated with 8 g dry wt/l activated dry yeast. Eight different temperatures were tested, ranging from 15°C to 40°C. Replica sets of inoculated and uninoculated flasks were placed in incubators and allowed to come to temperature; then, 2 mg/l Cr(VI) was added to each flask. After a 2-h incubation, a 10-ml aliquot from each flask was filtered through a preweighed 0.45-μm filter. The pellets were rinsed, dried, and weighed for biomass, and the filtered supernatants were analyzed for Cr(VI).

Hexavalent chromium toxicity to yeast cells

We evaluated the effect of Cr(VI) concentration on yeast metabolism by measuring the carbon dioxide gas (CO_2) production. Six different concentrations of Cr(VI) were tested, ranging from 0 to 1000 mg/l. The initial cell concentration was 20 g dry wt biomass/l . The incubation time was 16 h at 20°C and the cells were provided 100 mM glucose as a carbon source. CO_2 gas production was measured in triplicate calibrated water columns.

Effects of glucose concentration on chromate removal

The effect of 50, 100, and 500 mM glucose concentration on Cr(VI) removal from ground water (pH 8.1) was evaluated in batch tests. The experiments were started with 8.25 g dry wt/l yeast and continued for 6 h. The experiment was started with freshly hydrated yeast cells; the Cr(VI) initial concentration was 129 mg/l, and the incubation temperature was 30°C. We measured Cr(VI) removal and biomass in the media periodically over 6 h time course.

Kinetic studies

Kinetic studies on the interaction between Cr(VI) and *S. cerevisiae* were performed under completely mixed conditions on a rotary shaker (New Brunswick Sci. Co., 120 rpm). Chromate reduction experiments were performed in filtered ground water (pH 8.1) containing Cr(VI) at concentrations ranging from 0.1-50 mg/l. The hexavalent chromium source was $K_2Cr_2O_7$. The inoculum was *S. cerevisiae* (5 g dry wt biomass/l) and the incubations were performed at room temperature (21°C) with 25 mM glucose. Samples were taken from the suspension and filtered immediately through 0.22-μm membrane filters to remove cells. The filtrate collected was analyzed by atomic absorption spectrophotometry for residual Cr(VI) concentrations. CC and DC controls were used.

Reactor materials balance

An analysis of the feed and outflow liquid, gas and solid streams was conducted for three reactor test sets: LC, CC and DC controls. The ground water was amended with 2 mg/l (40 mM) Cr(VI) for each test set. The reactor used in this study was a modified New Brunswick BioFloIII attached to a Neslab chiller and an E_h monitoring system that included a textronics AMZ amplifier for increasing the mV signal. Data were collected on a Mac SX using National Instrument LabView software. The reactor was allowed to come to steady state prior to inoculation; the testing period was 24 h, and pH, E_h, dissolved oxygen, and agitation were measured.

At the end of each experiment, the reactor contents were divided into vapor, aqueous, and solid phases. The vapor phase from the reactor was collected in humidified tedlar bags, and sent with air blanks and standards to a certified laboratory (Air Toxics Inc., Sacramento, California) for vapor analysis (gas chromatography and mass spectroscopy to identify all total ion chromatogram (TIC) peaks). The culture aqueous phase was filtered through a 0.22-μM cellulose acetate Millipore membrane filter. The filtrate was collected in Erlenmeyer vacuum flasks and sent to a certified laboratory (California Laboratory Services, Rancho Cordova, California) for general minerals, anions, nitrogen (organic and inorganic), drinking water metals, and EPA 624 analysis. The cell wash filtrate was analyzed for chromium at LLNL. The solid phase was the residual cell material remaining on the filter paper after aqueous phase collection. The cell mass was washed with analytical grade water. The cell mass and filter paper were analyzed for total chromium.

Results and discussion:

Effect of pH on Cr(VI) removal

We observed that the removal of Cr(VI) under varying pH conditions was different for live *S. cerevisiae* than for dead *S. cerevisiae*. The optimum pH for *S. cerevisiae* for removal of Cr(VI) from solution was near neutrality. Between pH values of 6.5 and 7, 100% Cr(VI) was removed from the ground water sample (Table 3). The LC and DC cultures showed opposite results over a pH range of 2 through 8. In more acidic conditions, the Cr(VI) removed by live *S. cerevisiae* became less efficient while at the natural pH of MW-004 ground water (8.2), 67% of the Cr(VI) was removed. In contrast, dead *S. cerevisiae* in ground water with a pH of 8.0 removed only 0.5% of the Cr(VI) but at a pH value of 2.1, 70% of the Cr(VI) was removed from the filtrate (Table 4). In the DC controls, there was an inverse relationship of pH and Cr(VI) removal between pH 2 and 8. The proportion of absorption versus abiotic effects was not determined. Processes that may remove chromate from solution include absorption of Cr(III) by biomass, abiotic reduction of Cr(VI) at low pH, or enzymatic reduction of Cr(VI) by *S. cerevisiae*. *S. cerevisiae* grow actively at pH values lower than those optimal for most bacteria. Media with pH values of 2.0 to 8.0 did not significantly change the biomass of LC cultures over the 24-h test period.

Effect of temperature on Cr(VI) removal by S. cerevisiae

The influence of temperature on Cr(VI) removal rates in the presence of *S. cerevisiae* is shown in Figure 1. The optimum Cr(VI) removal rate occurred with incubation temperatures of 25° to 35°C. With a starting concentration of 10 mg/l Cr(VI) and 8 g/l cell dry mass per volume, the shake flasks that were maintained at 15°C resulted in a Cr(VI) reduction rate of 0.037 mg hr^{-1} (g dry wt. biomass)$^{-1}$ compared to 0.094 mg hr^{-1} (g dry wt. biomass)$^{-1}$ from test flasks maintained in 25°C. The maximum rate of 0.107 was obtained from the test flasks held at 35°C. Other authors have site increased metal uptake with elevated temperatures. Brady and Duncan (1994) studied the accumulation of copper with *S. cerevisiae* and determined that 25° to 30°C was the optimum temperature for maximum metal removal. The optimum temperature for uranium by *S. cerevisiae* was 50°C (Strandberg et al., 1981).

S. cerevisiae sensitivity to Cr(VI) concentrations

The data presented in Table 5 suggest that *S. cerevisiae* is very chromate tolerant. Unacclimated yeast cells metabolic activity did not decline, as measured by production of $CO_2(g)$ until concentrations of chromate exceeded 50 mg/l. Concentrations of chromate of 250 mg/l decreased $CO_2(g)$ production from an initial level of 4.8 down to 3.7 mg/l. The total amount of Cr(VI) removed from solution per gram of dry weight of biomass increases as the initial concentration of Cr(VI) increases; conversely, the percentage of the total Cr(VI) removed declines with higher concentrations of Cr(VI) (Table 6). A similar effect was noted by Brady & Duncan (1994). In Cu^{2+} bioaccumulation experiments they determined that the accumulation by yeast was dependent on the ratio of external free metal ion concentration to the available biomass.

Effect of glucose concentration on the removal of Cr(VI)

A glucose concentration of 100 mM in yeast cultures resulted in the best Cr(VI) removal rate from samples taken during 6 h of cell contact time (Table 6). Cr(VI) removal followed two-step kinetics. The live yeast cells removed approximately 55 mg Cr(VI) h^{-1} (g dry wt biomass)$^{-1}$ in the first two minutes (Table 6). After the initial uptake, the subsequent Cr(VI) removal requires glucose and occurs at a much slower rate. The initial accumulation is probably due to biosorption of Cr(VI) cations to the biomass, possibly the cell wall. The second, slower phase may be due to metal internalization and be enzyme dependent.

Rate of chromate removal

A double reciprocal plot of rates of chromate removal attained by *S. cerevisiae* during 0-order growth is shown in Figure 2. The double reciprocal plot is the rate of chromate reduction by yeast cells as a function of initial chromate concentration in the flask. *S. cerevisiae* removed hexavalent chromium in the form of chromate at concentrations ranging from 0.1 to 50 mg/l. The experiments were performed under completely mixed, anoxic conditions, using glucose (100 mM) as a carbon source. The rate of chromate reduction attained during complete chromate removal was 0.227 mg Cr(VI) h^{-1} (g dry wt biomass)$^{-1}$ and K_m at 145 mg/l^{-1} chromate. The high K value indicates that there is not a strong affinity for Cr(VI). Our results agree with a rate of Cr(VI) reduction of 0.2 mg Cr(VI) h^{-1} (g dry

wt biomass)$^{-1}$ (Mehlhorn et al. unpublished) obtained by using an electron spin resonance (ESR) technique (Mehlhorn et al. 1993).

We compared *S. cerevisiae* Cr(VI) removal rate to *P. fluorescens* (isolated from LLNL soils contaminated with BTEX). The *P. fluorescens* used in our test conditions, using ground water and environmental temperatures, was significantly slower than *S. cerevisiae* (initial rate of removal 0.47 μg h^{-1} (g dry wt biomass)$^{-1}$ versus 0.227 mg h^{-1} (g dry wt biomass)$^{-1}$), although other species of *P. fluorescens* may show different Cr(VI) removal rates. Bacterial kinetic studies revealed chromium removal rate vary with experimental conditions. A first-order constant of chromium removal of 0.1518 h^{-1} for an initial concentration of 1,000 ppm was determined in a study using a consortium of sulfate-reducing bacteria (Fude *et al.* 1994) and V_{max} of chromium(VI) reduction of 4.01 mg hr^{-1} (g dry wt. biomass)$^{-1}$ was determined in a study using *P. aeruginosa* PA01 (Apel & Turick 1992). Fude et al. (1994) concluded that the rate of Cr(VI) reduction depended on the concentration of Cr(VI) in the culture vessel.

Initially, *S. cerevisiae* rapidly removed Cr(VI) from the solution. Our studies suggest that this is followed by a significantly slower metabolically mediated Cr(VI) reduction (Table 6). In our bioreactor studies, we found between 45 and 52% of the Cr(VI) was removed from solution by the dead biomass. Mehlhorn et al. (unpublished results) has shown that a regime of freeze-thawing the yeast cells greatly enhanced their ability for Cr(VI) reduction. Both processes, the autoclaving and freeze-thawing, break cell membranes and may increase additional nonspecific binding of the chromium ion.

Results of reactor experiments

Effects of aerobic versus anaerobic reactor condition on Cr(VI) removal

A series of reactor tests were conducted to measure the effect of air on the cell's ability to remove Cr(VI) from solution. In one test, air was introduced to the reactor at 1.7 l/min, the maximum allowed by the reactor. The rate of Cr(VI) reduction/accumulation over time in the aerated test was compared to a reactor test in which no additional oxygen was provided. Both tests were conducted at the ground water temperature (19°C). The reduction of Cr(VI) in the oxygen-limited reactor was approximately 25% greater than in the reactor provided with continuous filtered air (Fig. 3). The initial pH was 8.42 in the aerobic culture. After 2 h the pH declined to 6.32 and after 24 h the pH

was 5.35. The culture grown without the addition of air also began with a pH of 8.42. After the 24 h test the pH was 4.55.

Materials balance of a batch reactor

Although many chemical reactions take place in the reactor, only the elemental ratios of chromium and carbon compounds in the effluent changed greatly. These byproducts depend on the level of chromium reduced, the heavy-metal concentration in the feed, and reactor pH, provided that optimal microbial conditions exist within the reactor. Microorganisms consume organic compounds, such as sugar, for growth and energy; a byproduct of this metabolism is the production of electrons. These electrons are used in the reduction of chromate to chromium hydroxide ($Cr(OH)_3$; the solubility is 6.7×10^{-31} mg/l).

The overall reaction in an anaerobic, acidic environment with glucose provided as the carbon source can be represented by:

$$8H^+ + 2H_2O + (CH_2O)_6 + 4Cr_2O_7^{2-} \longrightarrow 6CO_2 + 8Cr(OH)_3$$

In our materials balance study we examined both hexavalent and total chromium. Although we did not look at specific oxidative states, we assumed that in our setup, the chromium existed in two oxidative states: hexavalent and trivalent. Hexavalent chromium is the species we placed into the culture and is colored (yellow) in the parts per million range. Trivalent chromium is the thermodynamically preferred state in the acidified and unacidified samples. Note that we only assayed total Cr and hexavalent Cr species.

The CC culture hexavalent chromium concentration did not change over the 24 h test period (Fig. 4a). The CC culture total chromium concentrations were about 2 mg/l throughout the test and chromium in the reactor was in the hexavalent state. Before the addition of the DC biomass, the hexavalent chromium concentration was 2.1 mg/l (Fig. 4b). With the addition of the DC culture, the Cr(VI) concentration dropped to 1.0 mg/l. After the 24 h incubation the Cr(VI) concentration dropped to 0.58 mg/l. With the introduction of the dead-cell biomass, the total chromium concentration dropped to 1.1 mg/l and after 24 h the concentration was 0.94 mg/l. In the DC culture effluent 55% of the total chromium was removed, most likely due to biosorption. Strandberg et al. (1981) found an

increased rate of uranium uptake by dead *S. cerevisiae* biomass and suggested that it was due to surface-associated biosorption.

Hexavalent chromium in the LC culture was completely removed (Fig. 4c). However, only chromium species other than Cr(VI) remained in solution representing 30% of the initial chromium in solution. The LC culture absorbed only 8% of the chromium immediately (92% of the chromium was recoverable from the aqueous phase at 0 h). Depuration of the reduced metal from surface binding sites, cell lysis, or Cr(VI) reduction by extracellular enzymes, are possible explanations for chromium present in the aqueous phase after 24 h of cell contact. Another possibility is elution with sodium carbonates and bicarbonates. In studies conducted by de Rome and Gadd (1991), *S. cerevisiae* desorbed 60 to 80% of cesium or strontium when the loaded biomass was eluded with 1 M solution of sodium carbonate/sodium bicarbonate. Possibly, the high concentration of bicarbonates in our ground water eluded the reduced chromium from the biomass. Future research concerning extra- and intracellular chromium oxidative states and fates is needed.

The pH in the CC cultures was relatively constant, ranging from 8.2 to 8.3 without any need for adjustment. The reactor solution in the presence of LC culture has an initial pH of 8.1 but then declines to 2 or 3 due to the fermentation of glucose, which produces acids such as lactic acid. However, for the LC culture we controlled the reactor pH to 6.5 with 4 M sodium hydroxide. This decision was based on minimum pH values permitted for ground water surface discharge. The pH value of 6.5 was also chosen because yeast cells grow best at neutral pH values and in our studies the optimum Cr(VI) removal occurs when the culture is maintained at neutral pH values (Table 3 & 4). The pH in the DC cultures was about 8.3 at 0 h and dropped to 6.4 by 24 h due to bacterial contamination after about 15 h. Evidence of bacterial contamination in E_h, pH, and dissolved oxygen measurements occurred, and bacteria were found under microscope examination. We cultured these bacteria and spiked the culture with Cr(VI). This bacterial culture did not reduce Cr(VI) to Cr(III) or remove Cr(VI) by biosorption.

The redox potential (E_h) is determined by electron activity; matter in solution may either absorb or release electrons. Redox potential will increase with the addition of dichromate, a strong oxidizer, to ground water. Fude et al. (1994) found E_h increases by approximately +150 mV with increasing Cr(VI) concentration up to 1,000 mg/l. Local unamended ground water had a pH of 8.2 and an E_h of +343 mV at 20°C, a substantial oxidizing potential. A study of total chromium and Cr(VI) in ground water from 39 LLNL monitoring wells was conducted by W. W.

McNab and T. N. Narasimhan (unpubl.). Data from these analyses suggested that the average contribution to total chromium from Cr(VI) was virtually 100% due to the pH and E_h conditions of the ground water.

Data from LC inoculated reaction vessels suggested that reducing potential increased after approximately 5 h of acclimatization (Fig. 5). Reducing conditions developed only in the presence of viable cells. The chemical control redox potential maintained E_h at a relatively stable +286 to +307 mV through the 24 h test period. The heat-killed cells increased E_h from +345 to about +336 mV. The DC culture E_h stabilized at valve greater than the CC culture. The dead-cell biomass may have become an electron donor thereby increasing the E_h. The majority of Cr(VI) removal from the DC solution was likely due to biosorption and not reduction. However, 30% of the total chromium remaining was in an oxidated state other than Cr(VI), presumed to be Cr(III). In the LC culture E_h began at +321 mV and decreased to +90 mV during the 24 h test period. The pH was not allowed to decrease below 6.5 and most likely the addition of NaOH decreased the ability of the LC culture to reduce Cr(VI). Viable sulfate-reducing bacteria cultures developed reducing conditions (between +100 to +200) in experiments conducted by Fude et al. (1994). A solution with a negative E_h is capable of reducing Cr(VI) to Cr(III).

The initial ground water bicarbonate ion concentration was 150 mg/l. In the DC, the bicarbonate concentrations decreased to 73 mg/l by 24 h and increased to 3400 mg/l in the LC culture. Equilibrium between HCO_3^- and CO_3^{2-} is influenced by cellular CO_2 produced during the metabolism of glucose; CO_2 dissolves in water as carbonic acid (H_2CO_3). The formation of H_2CO_3 (a very weak acid) and its salts is strongly influenced by the prevailing pH. The carbonate-bicarbonate equilibria in the bioreactor is an incidental consequence of metabolic processes that affect pH. The pH in most media is controlled by a combination of dissolved gases (bicarbonate buffer system) and products of metabolism by the cell (especially lactic acid). As the pH decreases the equilibrium shifts towards bicarbonate production.

Specific conductance values changed significantly among the various groups (Table 7). Specific conductance, or the ability of a solution to conduct a current, depends on the presence of ions, their total concentration, mobility, valence, and relative concentration. The CC culture specific conductance value was about 850 µmho/cm (25°C), typical for chromate containing ground water. The DC culture specific conductances were 980 and 960 µmho/cm at 0 and 24 h, respectively. This increased conductivity may be due to lysed cells. The LC

culture specific conductance values were 840 and 5700 μmho/cm at 0 and 24 h, respectively. The increase in the specific conductance in the LC culture indicates an increase in electrolyte production.

Cells or cellular metabolic products altered the mineral equilibrium as measured by water hardness in the CC culture from 210 mg/l to 190 and 180 mg/l at 0 and 24 h, respectively, in the DC culture. The values in the LC culture were 170 and 150 mg/l at 0 and 24 h, respectively, a 28% decline in water hardness. Calcium concentrations in the CC culture were similar to those found in the ground water, whereas the values obtained for the LC and DC cultures were 30% lower than ground water values (Table 7). Magnesium concentrations in the CC, DC, and LC cultures were also similar to those found in the ground water (between 15 and 20 mg/l, Table 7). Barium concentrations in the CC and DC cultures were unchanged during the test. However, the barium concentration in the LC culture was decreased 15% during the test. Apparently, there is some affinity between the cells and barium. The potassium ion concentration changed significantly among the various groups.

The aqueous phases from all cultures were analyzed for standard drinking water metals: arsenic, barium, cadmium, hexavalent chromium, total chromium, lead, selenium, and silver (Table 7). Arsenic, cadmium, selenium, and silver were not detected at or above their levels of detection (LODs) in any test culture. In addition the aqueous phases from all cultures also were analyzed for the following anions: bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate. Bromide, nitrite, and phosphate were not detected at or above their LODs in any culture.

Summary

A potential solution to Cr(VI) contamination in ground water is to selectively remove the chromium with common baker's yeast, *S. cerevisiae*. We investigated the use of *S. cerevisiae* as an agent for reducing Cr(VI) to less toxic chromium oxidative states. To determine the optimum environmental parameters for *S. cerevisiae* metal reduction, we evaluated such factors as pH, dissolved oxygen, temperature, and nutrient levels. In bioreactor studies, the chromium removal rate was slightly faster under anaerobic, rather than aerobic, conditions. To determine the tolerance of the yeast culture to Cr(VI), organisms were exposed to concentrations up to 1000 mg/l. We obtained an overall mass balance by analyzing the feed and outflow liquid, gas, and solids in a stirred-tank reactor. A control test, conducted in the absence of microorganisms, resulted in 100% recovery of the Cr(VI) in the reactor liquid

effluent. Inclusion of *S. cerevisiae* in these experiments resulted in reduction of Cr(VI) to less toxic chromium cations. Cr(VI) reduction mediated by *S. cerevisiae* was verified by an electron spin resonance technique.

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Figure 1. Effect of ambient temperature on the bioaccumulation of Cr(VI) by yeast after a 2-h exposure. Experimental conditions: Cr(VI) concentration 10 mg/l; cell concentration was 8 g/l (cell dry mass per volume); initial pH 8; in ground water.
Second-order polynomial curve fit $Y = -0.73 + 0.09\ x + -0.001248x^2$ $R = 0.90$.

Figure 2. Double Reciprocal Plot of Cr(VI) reduction by *S. cerevisiae:* $V_{max} = 0.227$ mg hr^{-1} (g dry wt. biomass)$^{-1}$, $K_m = 145$ mg/l^{-1}. $Y = 4.41 + 642.29x$, $R = 0.99$.

Figure 3. The effect of air on the cell's ability to accumulate Cr(VI). Two reactor test were conducted with an initial reactor composition of 10 g/l biomass, 100 mM glucose, and 2 mg/l Cr(VI). The aerated culture was initially saturated with oxygen, and throughout the run the oxygen level was controlled at 1.7/l/min with sterile filtered air at 19°C. The initial pH and E_h were 8.42 and 312 mV, respectively.

Figure 4 A, B, C. Materials balance over bioreactor; A, chemical control; B, dead cell control; and C, live cell test.

Figure 5. Local ground water had a pH of 8.2 and an E_h of +343 at 20°C. Redox measurements were electronically recorded in the reactor at one-minute intervals during three 24-h tests. The tests included a chemical control, a killed-cell, and the *S. cerevisiae* test series. The redox potential readings are reported relative to the normal hydrogen electrode corresponding to the filling solution used and the temperature of the solution measured.

Table 1. MW-004 ground water inorganic and mineral analysis.

Analyte	Result (mg/l)
Barium	0.070
Bicarbonate alk (as $CaCO_3$)	280
Boron	1.400
Calcium	60
Chloride	120
Chromium (Total)	0.032
Copper	0.009
Hexavalent chromium	0.030
Iron	<0.01
Magnesium	20
Nitrate plus nitrate	20
Potassium	1.700
Sodium	120
Specific conductance-umhos/cm	1000
Sulfate	33
Total dissolved solids	560
Zinc	0.020
pH	8.2

Table 2. MW-004 ground water volatile organic compound analysis by EPA method 601.

Analyte	Result	Detection level
	µg/l (ppb)	
TCE	44	>2.5
PCE	<0.5	>0.5
1,1-DCE	2.2	>0.5
1,2-DCA	<0.5	>0.5
Carbon tetrachloride	<0.5	>0.5
Chloroform	2.4	>0.5
Freon 113	<0.5	>0.5

Table 3. Cr(VI) removal by live*S. cerevisiae* biomass at several pH. *S. cerevisiae* concentration = 1.5 g/l, glucose concentration = 50 mM, Cr(VI) concentration = 2 mg/l in MW-4 ground water for a 24 h exposure. The pH was adjusted with 2 N HCl, and the ambient test temperature was 20°C.

Initial pH	Final pH	Initial [Cr(VI)] (mg/l)	Final [Cr(VI)] (mg/l)	Cr(VI) removal (%)
2.0	2.0	2.11	0.77	61
4.0	3.1	1.91	0.38	81
5.0	3.8	1.91	0.35	82
6.1	3.9	1.96	0.21	89
6.5	4.0	1.84	0.00	100
7.0	4.4	1.85	0.00	100
8.2	4.9	1.96	0.67	67

Table 4. Cr(VI) removal by dead *S. cerevisiae* biomass at several pH. Initial *S. cerevisiae* concentration = 1.5 g/l , glucose concentration = 50 mM, autoclaved for 35 min at 121°C; Cr(VI) concentration = 2 mg/l in MW-4 ground water for 24-h exposure. The pH was adjusted with 2 N HCl, and the ambient test temperature was 20°C.

Initial pH	Final pH	Initial [Cr(VI)] (mg/l)	Final [Cr(VI)] (mg/l)	Cr(VI) removal (%)
2.1	2.0	2.11	0.60	70
4.0	4.1	1.91	1.35	32
6.0	5.9	1.96	1.61	20
8.0	7.8	1.96	1.97	0.5

Table 5. Effect of Cr(VI) concentration on the carbon dioxide gas production and Cr(VI) removal rate by *S. cerevisiae*. The cell concentration was 20 g/l (cell dry wt per volume). The incubation time was 16 h, and the cells were provided 100 mM glucose as a carbon source.

Cr(VI) (mg/l)	CO_2 produced (ml/h) (SD)	Biomass concentration (mg/l) (SD)	Cr(VI) removed (mg/h/g) biomass (SD)	% Removed (SD)
0	4.8 (0.3)	7.82 (0.2)	0.00 (0)	0.00 (0)
5	5.1 (0.5)	8.15 (0.2)	0.19 (0.0)	94.48 (3.4)
50	4.9 (0.3)	8.25 (0.3)	0.92 (0.1)	52.26 (11.9)
250[1]	3.7 (0.3)	—	—	—
500	2.4 (0.4)	8.27 (0.3)	2.56 (1.8)	12.37 (8.2)
500[1]	2.6 (0.7)	—	—	—
1000[1]	2.1 (0.3)	—	—	—

[1] 200 mM glucose provided to cultures.

— analysis not available

Table 6. Cr(VI) removal rates with 50, 100, and 500 mM glucose in shake flasks. Experimental conditions: 30°C; initial Cr(VI) concentration of 129 mg/l; 8 g/l dry wt of freshly hydrated *S. cerevisiae* inoculum; initial pH 8.1 in ground water in culture vessels. Removal of Cr(VI) mg/h/g dry wt biomass was calculated by dividing Cr(VI) mg removed from the supernatant by the g dry wt biomass. The results are the average of replicate samples.

Glucose (mM)	Biomass (g/l)	Time (h)	Cr(VI) mg removed/h	Removal of Cr(VI) mg h^{-1} (g dry wt biomass)$^{-1}$
50	8.1	0.00	—	—
	8.3	0.03	456	54.94
	8.3	0.50	5.20	0.63
	8.3	1.00	22.9	2.76
	8.4	2.00	12.0	1.43
	8.5	3.00	8.27	0.97
	8.7	5.67	5.21	0.60
100	8.1	0.00	—	—
	8.1	0.03	444	54.81
	8.1	0.50	59.6	7.36
	8.2	1.00	33.0	4.02
	8.2	2.00	17.4	2.12
	8.2	3.00	15.1	1.84
	8.4	5.67	9.4	1.12
500	8.1	0.00	—	—
	8.6	0.03	552	64.19
	8.7	0.50	43.2	4.97
	8.8	1.00	21.2	2.41
	9.1	2.00	15.1	1.65
	9.4	3.00	13.5	1.44
	10.1	5.67	8.4	0.83

Table 7. Aqueous phase general mineral and metals data for the chemical control, dead cell culture, and live cell culture at 0 and 24 h. The matrix was a MW-4 ground water spiked with 2 mg/L (ppm) hexavalent chromium. All units are reported in mg/l.

	Chemical Controls		Dead Cell Culture		Live Cell Culture	
Analyte	Time 0	Time 24	Time 0	Time 24	Time 0	Time 24
Calcium	52	52	38	37	35	34
Magnesium	20	20	23	22	20	15
Potassium	3.0	3.2	32	30	10	7.0
Chloride	120	120	200	190	130	120
Flouride	0.45	0.46	0.43	0.40	0.43	0.42
Specific Conductance	850*	880*	980*	960*	840*	5700*
Arsenic	ND**	ND	ND	ND	ND	ND
Barium	0.075	0.073	0.069	0.070	0.067	0.057
Cadmium	ND	ND	ND	ND	ND	ND
Lead	ND	ND	ND	ND	ND	0.002
Selenium	ND	ND	ND	ND	ND	ND
Silver	ND	ND	ND	ND	ND	ND

* Units are μmho/cm at 25° C.

** Not detected at or above the limit of detection.

LODs are (mg/L): Calcium 1.0 (EPA 200.7); Potassium 1.0 (EPA 200.7); As 0.002 (EPA 206.2); Ba 0.025 (EPA 200.7); Cd 0.0005 (EPA 213.2); Pb 0.002 (EPA 239.2); Se 0.002 (EPA 270.2); Ag 0.001 (EPA 272.2).

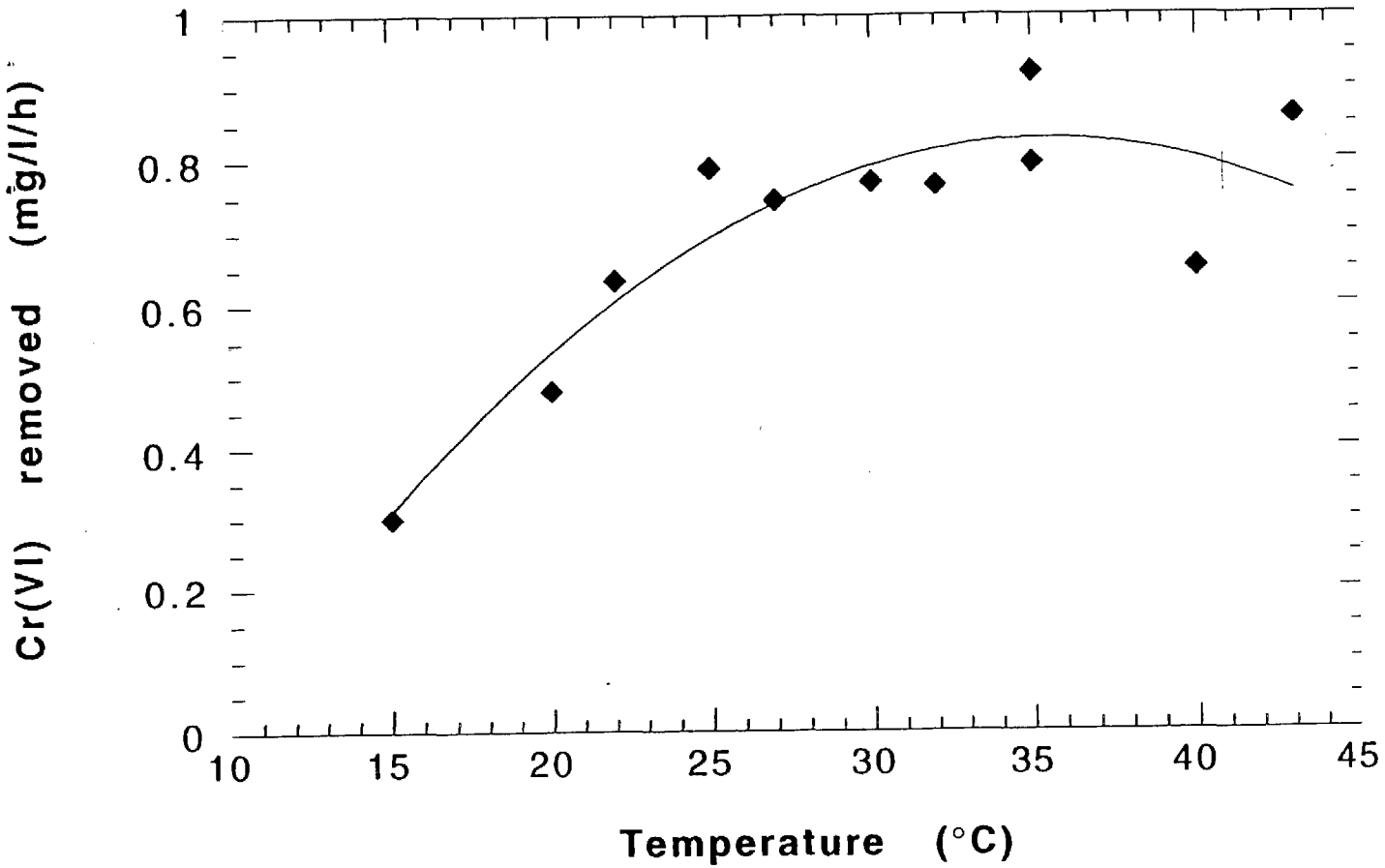

Cr(VI) removed (mg/l/h)
1
0.8
0.6
0.4
0.2
0
10
15
20
25
30
35
40
45
Temperature (°C)

fig 1

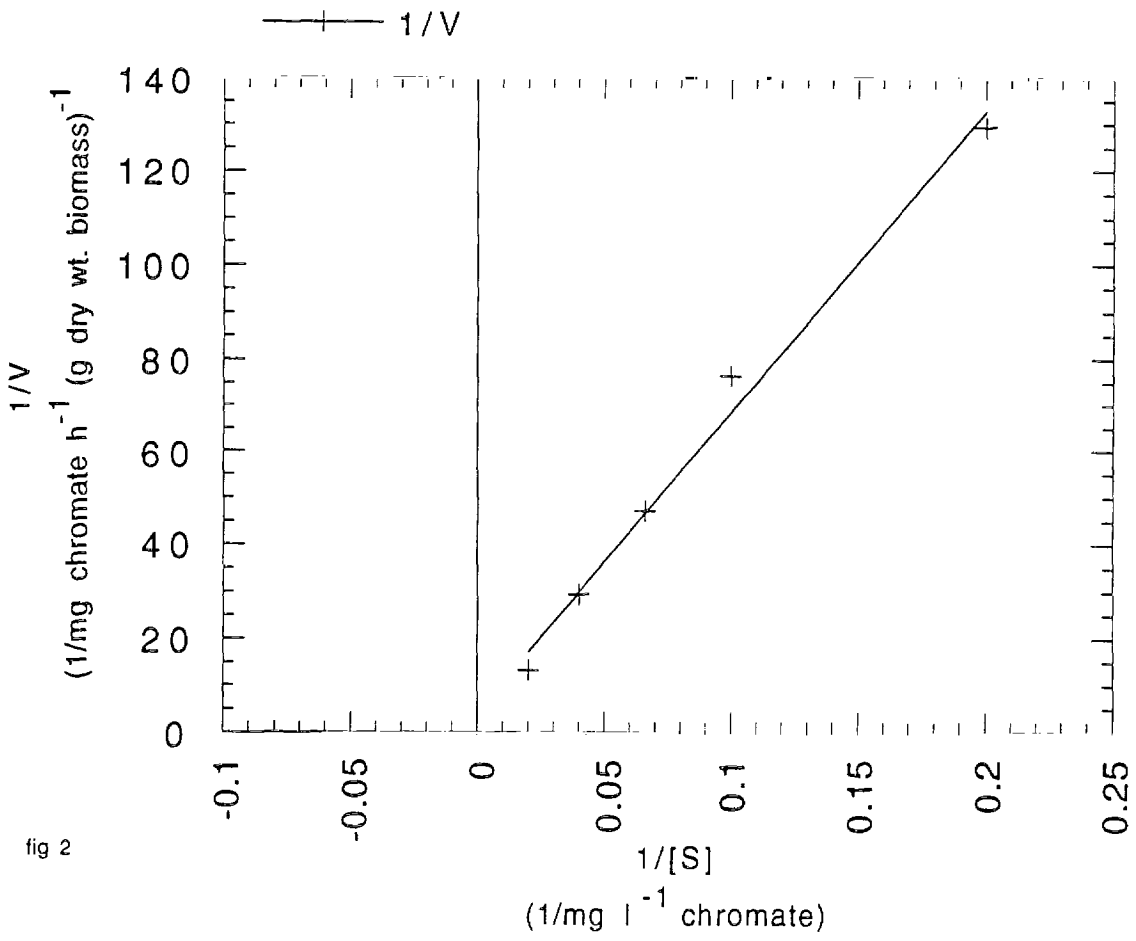

1/V
140
120
100
80
60
40
20
0
1/V
(1/mg chromate h-1 (g dry wt. biomass)-1
-0.1
-0.05
0
0.05
0.1
0.15
0.2
0.25
1/[S]
(1/mg l-1 chromate)

fig 2

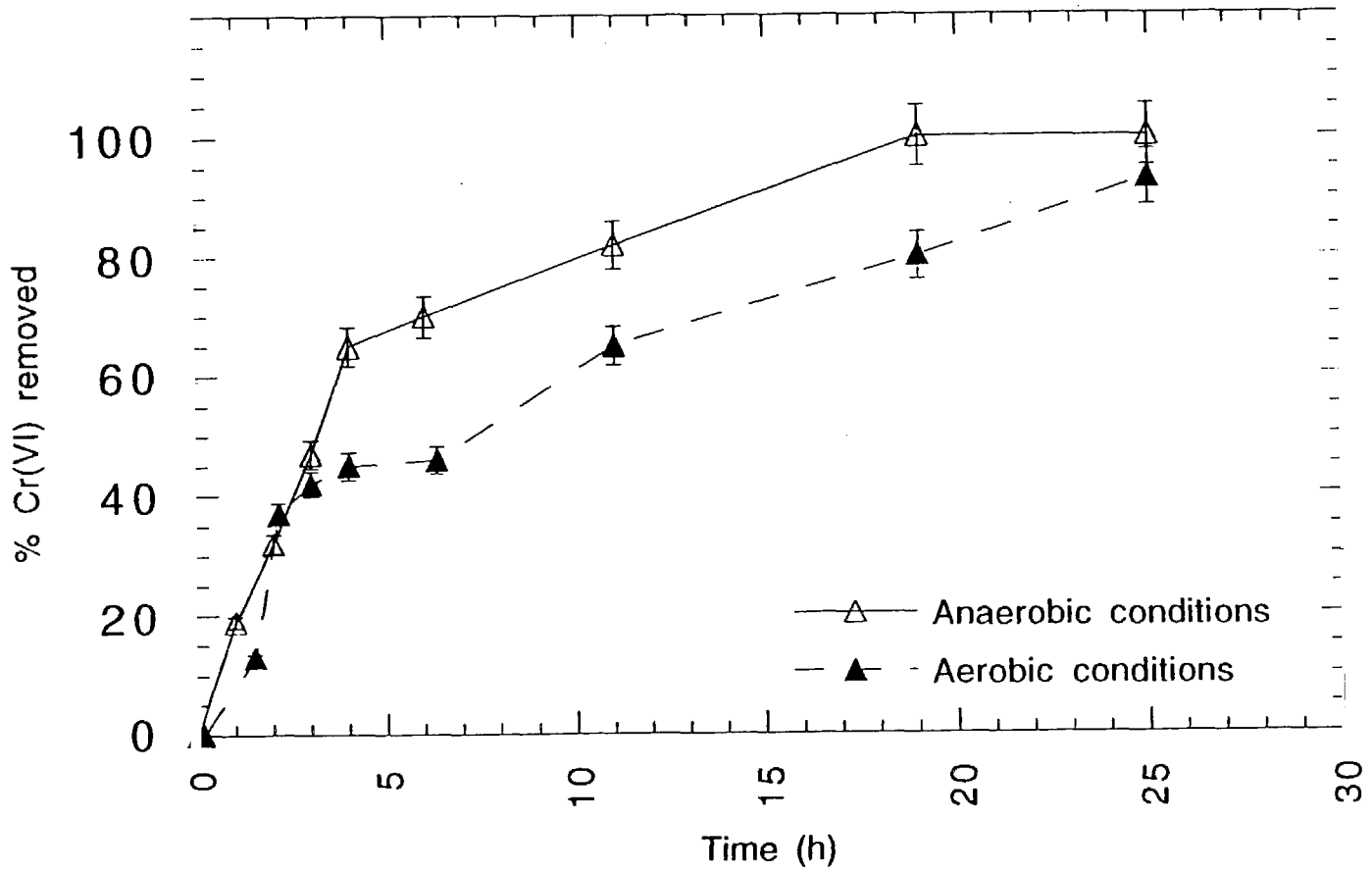

% Cr(VI) removed
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (h)
Anaerobic conditions
Aerobic conditions

fig 3

I. Chemical control

Gas Effluent

Ethanol (ppmv)	1.5
Carbon dioxide (%)	0.05

Ground Water

Hexavalent chromium (mg/l)	2.08
Total chromium (mg/l)	2.1
Bicarbonate (mg/l)	270
Dissolved oxygen (%)	99
E_h (mV)	310
Hydroxide ion (mg/l)	< 0.1
Iron (mg/l)	< 0.01
Lactate (mM/l)	< 0.2
Nitrate (mg/l)	5.1
pH (units)	8.2
Sulfate (mg/l)	53
Total dissolved solids (mg/l)	5000

BIOREACTOR

Glucose (mM/l)	54
Biomass $(CH_2O)_6$ (mg/ml)	0

24°C
~1 atm
pH 8.2

Aqueous Effluent

Hexavalent chromium (mg/l)	2.1
Total chromium (mg/l)	2.3
Bicarbonate (mg/l)	260
Dissolved oxygen (%)	108
E_h (mV)	307
Hydroxide ion (mg/l)	< 0.1
Lactate (mM/l)	< 0.2
Glucose (mM/l)	54
Nitrate (mg/l)	5.0
pH (units)	8.29
Sulfate (mg/l)	53
Total dissolved solids (mg/l)	4900

Solid Effluent

Biomass $(CH_2O)_6$ (mg/ml)	0.0
Total chromium (mg/l)	0.0

II. Dead-cell control

Gas Effluent

Ethanol (ppmv)	14
Carbon dioxide (%)	1.7

Grourrd Water

Hexavalent chromium (mg/l)	2.1
Total chromium (mg/l)	2.1
Bicarbonate (mg/l)	150
Dissolved oxygen (%)	100
E_h (mV)	345
Hydroxide ion (mg/l)	<0.1
Iron (mg/l)	<0.01
Lactate (mM/l)	<0 2
Nitrate (mg/l)	5.5
pH (units)	8.3
Sulfate (mg/l)	61
Total dissolved solids (mg/l)	5200

BIOREACTOR

Glucose (mM/l)	50
Biomass $(CH_2O)_6$(mg/ml)	1.05

24°C
~1 atm
pH 6.5

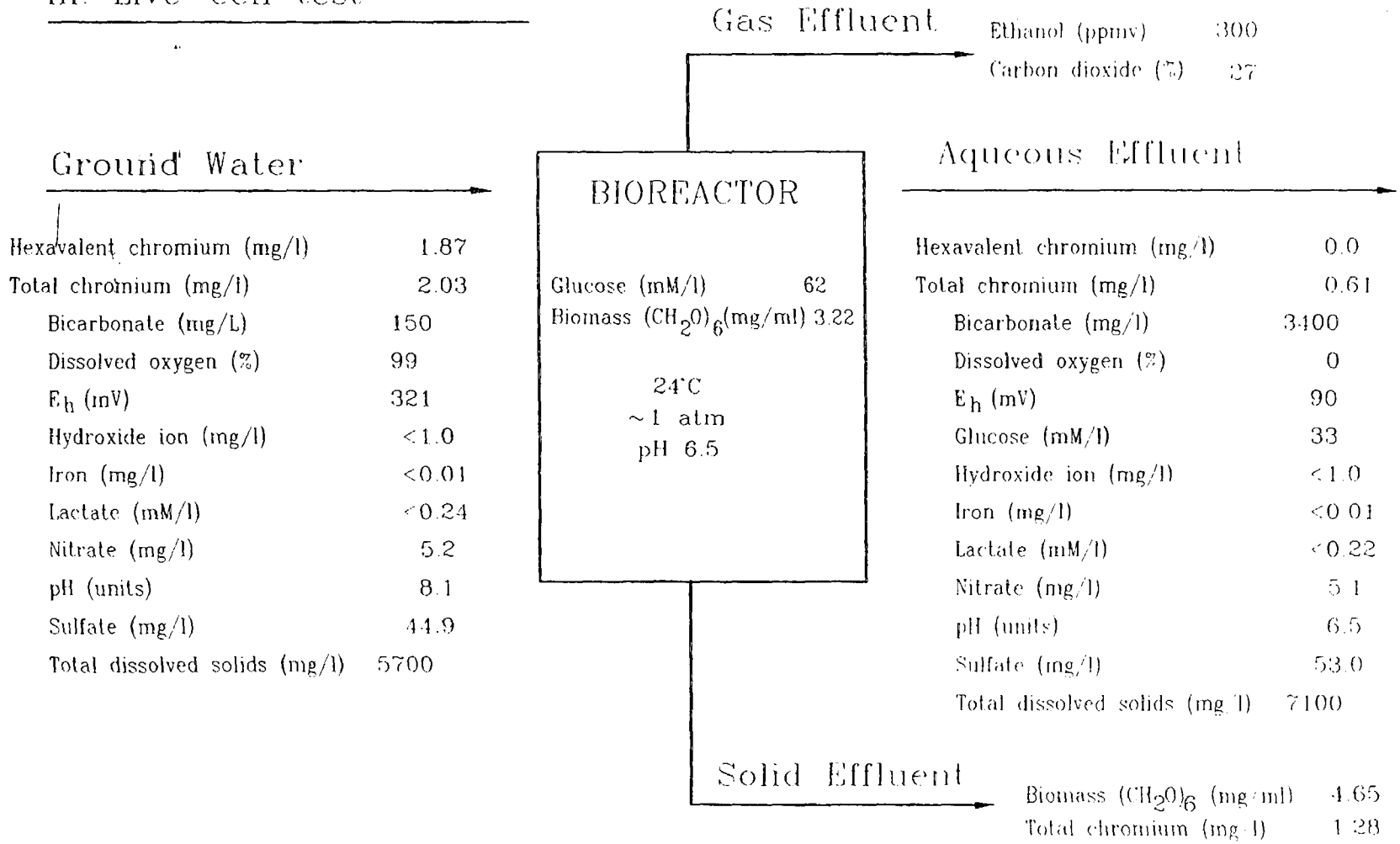
Aqueous Effluent

Hexavalent chromium (mg/l)	0.58
Total chromium (mg/l)	0.94
Bicarbonate (mg/l)	73
Dissolved oxygen (%)	0
E_h (mV)	336
Glucose (mM/l)	54
Hydroxide ion (mg/l)	<0.1
Lactate (mM/l)	4 5
Nitrate (mg/l)	5 4
pH (units)	6.0
Sulfate (mg/l)	62
Total dissolved solids (mg/l)	5000

Solid Effluent

Biomass $(CH_2O)_6$ (mg/ml)	1.13
Total Chromium (mg/l)	0.68

III. Live-cell test



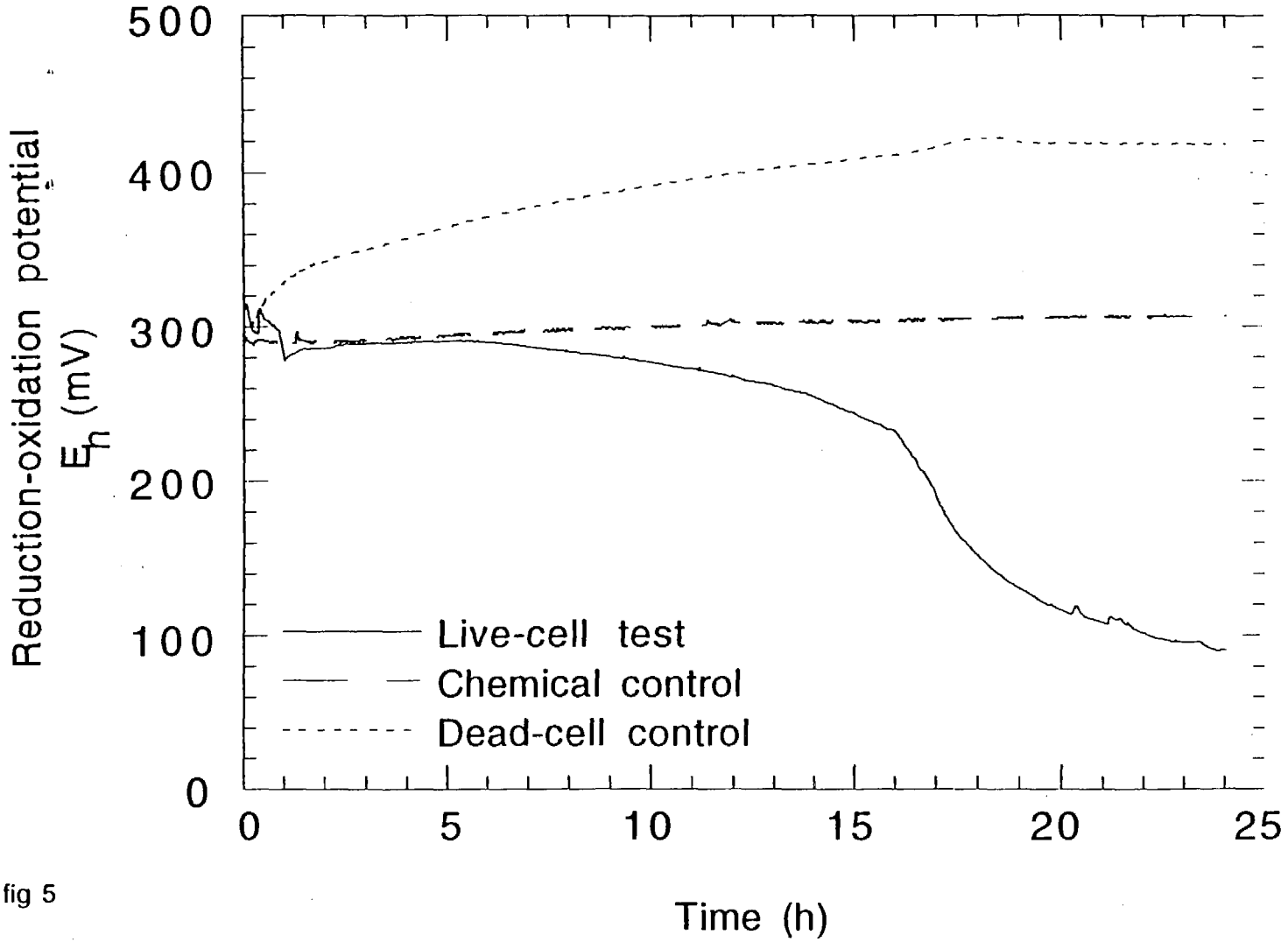

Reduction-oxidation potential
E_h (mV)
500
400
300
200
100
0
0
5
10
15
20
25
Time (h)
Live-cell test
Chemical control
Dead-cell control

fig 5